REMARKS/ARGUMENTS

I. Status of the Claims

Claims 108-118 are pending. Claim 119 has been cancelled without prejudice or disclaimer. Applicants reserve the right to file one or more applications directed to the canceled subject matter.

Claim 108 has been amended to disclaim humans. Support is, *inter alia*, on page 8, lines 1-9, where human is only one possible embodiment. No new matter is added.

II. Rejection Under 35 U.S.C. §112, First Paragraph

On page 2 of the Office Action claims.108-119 are rejected under 35 U.S.C. §112, first paragraph, on the grounds that they fail to comply with the enablement requirement. Applicants respectfully traverse the rejection.

Page 4 of the Office Action states "the specification does not provide any specific guidance for introducing the recombinant cells into an animal and how and under what conditions will the cells be maintained in an animal such that the desired endogenous gene overexpresses the protein". Applicants submit that specific guidance was not necessary as will be discussed in this Response.

References Reporting Recombinant Protein Expression *In Vivo* Demonstrate That RAGE* Cells Would Also Have Been Useful to Express Protein *In Vivo*

The Office Action discusses references that Applicants previously submitted. These references were submitted to demonstrate that the art reported various non-therapeutic uses for protein expression from implanted cells. The references were submitted originally because, in the Office Action dated February 14, 2001, Examiner Brunovskis stated that the claimed method did not have a well-established non-therapeutic utility. See the Office Action dated February 14, 2001, pages 7, 8, and 10.

*In the present Office Action, the Examiner discusses these references as they relate to enablement. The Examiner states that the references would not have been relevant to RAGE cells *in vivo* because the references were directed to "specific methods for specific cells". Office Action, page 4. Applicants submit that these references are relevant because RAGE cells could have been put to the same uses.

The chart below lists all of the references, the cell type used, the gene product expressed, whether endogenous or exogenous, the host animal, and the mode of administration.

* The term "RAGE cell" is a convenient designation for Applicants' recombinant cells where an endogenous gene is activated by non-homologous insertion of a regulatory sequence.

	Cell	**Gene Product**	**Host**	**Mode of Administration**
Kints	Hybridoma	Ig[A]	Rat	IP
Kints	Immunocytoma	Ig[A]	Rat	IP
Brodeur	Hybridoma	Ig[A]	Mouse	IP
Stewart	Hybridoma	Ig[A]	Mouse	IP
Shaw	T cells	IL-4[B]	Mouse	IV
Chen	Fibroblasts	HGF[B]	Rat	Direct injection
Garver	NIH3T3	$\propto$IAT[B]	Mouse	IP
Ishihara	AH109A	Protease[A]	Rat	IP
Bronson	ESC	Bcl-2[B]	Mouse	Transgene
NcNeice	WEHI	IL-3[A]	Mouse	IP
6,692,737	Rabbit fibroblast	HGH[B]	Mouse	Direct injection src
6,692,737	Rabbit fibroblast	HGH[B]	Rabbit	Direct injection src
6,531,124	Human fibroblast	GLP-1[B]	Mice	Direct injection src
6,054,288	Rabbit fibroblast	HGH[B]	Mice	Direct injection src
6,054,288	Rabbit fibroblast	HGH[B]	Rabbit	Direction injection src

[A] – endogenous, not genetically engineered
[B] – exogenous

Shaw used a T-cell line (Phoenix Packaging Cell Line). Chen used primary fibroblasts. Garver used the NIH3T3 fibroblast cell line. Ishihara used the AH109A hepatoma cell line. Bronson used an embryonic stem cell line subcloned from the E14 parent cell. McNeice used the WEHI-3 myelomonocytic leukemia cell line. Three additional patents show rabbit or human fibroblasts.

The chart demonstrates that a variety of recombinant cells had been used to successfully express proteins *in vivo*. The chart also demonstrates that a variety of genes could be expressed successfully *in vivo*. This is relevant to RAGE cells because they would be capable of substitution for any of the reported cells. It would have been expected that a given desired gene could be expressed in RAGE cells and that RAGE libraries could be made using a given desired cell type, including any of those in the art. See, for example, Applicants' specification, pages 53 and 54, disclosing numerous potential host cells. In fact, Applicants have made RAGE libraries in a variety of host cells, including human HEK293, HT1080, H4, HCT116, A431, MCF-7, Jurkat, HH1-SK-N-HS, mouse cell lines, and R1 embryonic stem cells. Therefore, the RAGE cell could reasonably have been predicted to substitute for any of the recombinant cells in the art.

1. Exogenous Coding Sequences

Nine of these references report using *exogenous* coding sequences. The Examiner only discusses Chen and Garver. Both Chen and Garver introduced exogenous coding sequences. Chen introduced the exogenous coding sequence for nerve growth factor into rat fibroblasts. Garver introduced ∝-1 antitrypsin coding sequence into NIH3T3 mouse cell line.

The Examiner states that a cell expressing an exogenous gene is not the same as a cell expressing an endogenous gene. But there is no scientific basis given for this statement. Applicants submit that the fact that *in vivo* expression was achieved with the cells in the art is a reasonable predictor that *in vivo* expression would be achieved with RAGE cells. If the rejection

is maintained on this basis, Applicants request specific scientific reasons to support the conclusion.

In point of fact, gene therapy involving an endogenous coding sequence is less likely to be problematic than gene therapy with exogenous coding DNA. Kay (cited by Examiner) states that the most difficult obstacle to overcome is the inability to transfer the appropriate gene into a target non-germ tissue such that an appropriate amount of gene product is produced to correct the disease. It states that the cell/organism has developed powerful mechanisms to avoid the accumulation of extraneous genetic material (page 12744, column 1). In the instant case there is less extraneous genetic material because the gene to be expressed is endogenous.

2. Endogenous Coding Sequence

The chart below lists the three patents discussed above and adds four more relevant patents (underlined). The four added patents are directed to *in vivo* expression of protein from an *activated endogenous locus.* An exogenous transcriptional regulatory sequence is used to activate an endogenous gene, which is expressed *in vivo.*

This art is also relevant to the usefulness of RAGE cells. If endogenous gene expression occurs when a promoter is introduced by homologous recombination it would be expected to occur when a promoter is introduced by non-homologous recombination. Accordingly, unless there is scientific reason presented to the contrary, this art, at the very least, would lead an artisan to expect *in vivo* expression of a protein in RAGE cells.

Patent	File Date	Claim	Disclosure	Example
6,054,288	1991	1, 4	10:23-67 4:51-67 11:1-2	Nude mice/rabbit fibroblasts/hGH Rabbits/rabbit fibroblasts/hGH
6,692,737	1991	1, 4, 10	4:51-61 5:1-6 10:19-31 10:55-63	Nude mice/rabbit fibroblasts/hGH Rabbits/rabbit fibroblasts/hGH
6,531,124	1992	1, 4	11:35-67 4:46-67 12:1-5	Nude mice/rabbit fibroblasts/hEPO None for GLP (Prophetic)
6,537,542	1991	1	10:56-67 11:21-29	None
6,187,305	1991	1	10:56-67 11:21-31	None
6,048,524	1991-1992	1, 3	4:41-63 11:1-13 11:30-67	Nude mice/rabbit fibroblasts/EPO
5,968,502	1991-1992	42	13:58-67 2:60-67 14:1-10 3:1-46	None

Further Issues

In addition to discussing the references that have been cited, the Examiner raises three issues: introducing a cell, maintaining a cell, and expressing protein from a cell implanted *in vivo*. Applicants address these issues below.

1. Levels of Gene Expression/Gene Silencing

The Examiner cites Kay et al., Proc. Nat'l. Acad. Sci, 94:12744-12746 (1997) and Anderson, Nature 392:25-30 (1998). The Examiner states "[f]or example, recombinant hematopoietic stem cells transduced with retroviral vectors when introduced in an animal express the recombinant gene at a very low, undetectable levels [sic]". Neither Kay nor Anderson stand for this proposition. The problem discussed by Kay (12746 column 1) is the problem of *gene transfer* into hematopoietic stem cells using *viral* vectors. These particular cells are refractory to viral transduction because they lack the receptors to take up the virus. Thus, this "problem" is not reported as a problem of low levels of recombinant gene expression.

Anderson also focused on viral-based vectors. Anderson also discusses the primitive hematopoietic stem cell as being a desirable target for gene therapy but a very poor recipient for virus. This is because, as stated by Anderson on page 25, column 2, the hematopoietic stem cell has a low level of receptor for the virus.

Anderson also discussed problems with manufacturing the virus vectors in sufficient quantity. This is not relevant to the claimed method. Anderson also refers to the issue of long-term stable gene expression and states that it is perhaps the greatest shortcoming of viral vectors. He states, however, that this particular issue applies to gene transfer vectors of all types. Applicants point out, however, that despite this shortcoming, successful treatment of humans has

been achieved and many clinically-relevant animal models have been developed from genetically engineered cells with both exogenous and endogenous genes.

Applicants also point to successful clinical trials in humans (New England Journal of Medicine, 344:1735-1742 (2001)). Cells were obtained from hemophilic patients and transfected with sequences that encode Factor VIII. After propagation *in vitro* they were introduced into patients. Increased plasma levels of Factor VIII activity were observed in four of six patients. This correlated with a decrease in bleeding, a reduction in the use of exogenous Factor VIII or both. In the patient in whom the highest level was produced, these changes lasted for 10 months. Experiments in nude mice were used as the basis for carrying out these Phase I trials.

Therapeutic efficacy for EPO and hGH has also been shown in Example 10 of U.S. 5,994,127. See the attached Appendix. It demonstrates the efficacy of exogenous human EPO in mice rendered anemic by systematic bleeding. In that example, nude mice were implanted with rabbit fibroblasts transfected with the human EPO gene. Immunoreactive human EPO was readily detectable. Bled animals exhibited high blood hematocrit levels compared to control animals. This shows that fibroblasts engineered to express EPO can survive *in vivo* and prevent a drop in hematocrit.

Example 11 of U.S. 6,054,288 shows that fibroblasts successfully produce human hGH in rabbits for 11 months. This is comparable to the levels of hGH that circulate in the serum of normal humans who do not suffer from hGH deficiency.

Accordingly, this is direct evidence recombinant gene expression has been used successfully to provide three therapeutically distinct proteins in three different mammalian species.

Effective Expression *in Vivo*: Non-Human Animals

The claims are now limited to non-human animals. Applicants point out that even Anderson demonstrates that gene therapy was widely effective in non-human animals. In the introduction on page 26, column 1, Anderson states that more than 300 human clinical protocols were achieved between 1990 and 1998 and over 3,000 patients were carrying genetically engineered cells. Therefore, efficacy in animal models had to have been demonstrated to justify proceeding with human clinical protocols. The fact that so many human trials were approved is evidence of the effectiveness of gene therapy in non-human animals.

2. Introducing Cell

The process for introducing a cell into an animal by any of the various routes of administration has been known for decades. Cells have routinely been introduced into animals at various sites by known methods of administration, for example, renal subcapsular, subcutaneous, central nervous system (including intrathecal), intravascular, intrahepatic, intrasplanchnic, intraperitoneal (including intraomental or intramuscular). Accordingly, implantation, whether for therapeutic or non-therapeutic uses, could have been achieved by ordinary and well-known

procedures. The Examiner provides no evidence why the process of introduction would not have been known to the person of ordinary skill of the art.

Applicants refer the Examiner to the U.S. patents in the chart above. In each of the patents, claims are broadly directed to introducing recombinant cells to an animal, including human. The disclosure for methods of introduction is without any specific detail. It generally states what was already well-known as of 1991-1992. The state of the art, presumably, would have even been less-developed than it was when Applicants filed their earliest priority application (September 1997). The disclosure is cited in the chart. The actual text in an attached Appendix. The claims are not limited to any particular method of administration.

It is well known in the patent law that what would have been known to the person of ordinary skill in the art, without the Applicants' specification, does not need to be included and, indeed, should not be included in Applicants' specification. Accordingly, because methods of implanting cells were routinely known and used in the art, Applicants' specification is sufficiently enabled for this aspect of the invention.

3. Cell Maintenance *In Vivo*

On page 5 of the Office Action, the Examiner asserts that maintenance of xenogeneic or allogeneic cells was not enabled. The Examiner cites Gage, Nature 392:18-24, 1998 and Samstein et al, J. Am. Soc. Nephrol. 12:182-193, 2001.

Neither reference supports the general statement that maintaining xenogeneic or allogeneic cells was not enabled. It is correct that a cell implant could be subject to attack by the host's immune system. But at the same time, it was well-known in the art that the attack could be addressed by standard immunosuppressive regimens, use of barrier devices, use of MHC compatible (syngeneic) cells, autologous cells, or immuno-deficient animals. The person of ordinary skill in the art of cell therapy would have been aware of these available methods. The use of immunosuppressive chemical or biological agents, such as cyclosporine, has been known for at least 30 years. Barrier devices have also been used for nearly 40 years. Such barrier devices would have served the function of preventing the cells of the immune system from reaching the implanted cells while allowing products made by the implanted cells to exit the barrier device. Use of syngeneic cells has also been known for many decades (i.e., "tissue-typing").

Applicants again cite the patents in the chart above. There are no specific details for dealing with immune suppression. There is only a cursory general reference to immunosuppression and barrier devices, both of which were well-known even in 1991. This disclosure is cited in the chart. The actual text is provided in the attached Appendix. In spite of this cursory general disclosure, claims in six of the patents are not limited to autologous cells. Applicants submit that this is because general immunosuppressive or avoidance techniques were so well known in the art. Note that the art was less developed by 5 or 6 years than the state of the art at the time Applicants filed their first priority application.

The Examiner cites Samstein et al., directed to organ transplantation across species barriers. This presents special problems not encountered with recombinant cells. Physiologic considerations (i.e., organ physiology) do not apply. The problem of infection does not apply. A barrier device, practical with cultured cells, would not apply to organ transplant.

On page 187 Samstein states that immunosuppression is currently quite effective in preventing cellular rejection of allografts. The question that is posed in the article, but not answered, is whether the same immunosuppressive regimens will be adequate to prevent and/or treat cellular rejection of xenografts in humans. See column 2, page 187. Accordingly, this reference really is designed to discuss the possibility of using non-human organs in human subjects. It only poses the question of whether successful immunosuppressive techniques, successful for allogeneic organ transplant in humans, will be successful for xenogeneic organ transplant in humans. Human models have obvious ethical problems. Nonetheless, animal models of xenotransplantation were not an issue.

Applicants also point out that long term survival is not necessary for a therapeutic effect. For example, transfected xenogeneic or allogeneic cells could be used for short-term therapy such that the gene product produced by the cells will be delivered *in vivo* until the cells are rejected by the host's immune system. Another situation concerns an antigenic protein being delivered to an experimental animal to induce antibody production. Here, the use of cells that will ultimately be rejected (xenogeneic or allogeneic transfected cells) can be used to limit exposure to the antigen since antigen production will cease when the cells have been rejected.

Since methods of immunosuppression, immune avoidance, or using immunocompatible cells were well known in the art, it was not necessary for Applicants to disclose them in the specification. Accordingly, as to maintenance of xenogeneic, allogeneic, syngeneic or autologous cells, Applicants submit that these embodiments are enabled.

III. Summary and Conclusion

Gene Expression In Vivo

Cited references show many different instances where recombinant cells express effective amounts of a desired gene. It is reasonable to expect that RAGE cells would be equally effective. RAGE cells can express a variety of genes and can be made from many cell sources.

Immune System

The claimed method is enabled for xenogeneic, allogeneic, syngeneic or autologous host cells. Methods for tissue typing, immunosuppression or immune avoidance were widely known and used by persons of ordinary skill in the art. Applicants have presented numerous patents with broad gene therapy claims and marginal disclosure for immunity issues.

Amendment

Claims are now limited to non-human animals. The multitude of human clinical trials with recombinant cells evidences safety and therapeutic efficacy in animal models. Applicants have also provided direct evidence of efficacy of recombinant cells in animals.

Accordingly, Applicants believe that they have addressed each of the grounds of the rejection and the rejection has been overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

Respectfully submitted,

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